STUDIES IN RODENT POLIOMYELITIS

V. Interference between Murine and Monkey Poliomyelitis Virus*

By CLAUS W. JUNGEBLUT, M.D., and MURRAY SANDERS, M.D.

(From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York)

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The occurrence of synergism or antagonism between microorganisms or viruses is generally known to workers in the field. Even though the mechanism of these phenomena is by no means well understood, the fact that the course of an infectious disease may be significantly altered by the concomitant action and interplay of two different etiological agents, or their growth products, offers an attractive field for experimental investigation.

As far as bacterial antagonism is concerned, inhibitory effects are usually the result of a destructive action *in vitro* and *in vivo* of certain bacterial enzymes, like pyocyanase, gramicidin, and penicillin, upon certain microorganisms. Among the protozoa, a well defined mutual suppression of two invading parasites has been described for mixed infections of bartonella and eperythrozoon in mice (1). However, not until one comes to the field of virus diseases is interference found firmly established as a distinct biological phenomenon. Probably the first reference to domination of one virus by another was made in 1929 by McKinney (2) who reported that a yellow-mosaic virus, derived from the common light-green mosaic of tobacco, would not propagate in tobacco plants in which the common-mosaic virus was already present. Subsequent studies by McKinney (3) and others (4) have widened the scope of the interference phenomenon among plant viruses and added much to our knowledge of how to utilize this reaction as a possible means for establishing relationships between mutants and non-relationships between distinct viruses.

Examples of interference in virus infections of animals and man are not very numerous, nor have those on record received more than scant attention. What appears as evidence of crossed resistance has been described for certain virus systems, *i.e.* pseudorabies-virus B (5) and vaccinia-herpes (6); but such protection as may be observed in these cases is probably due to an overlapping group immunity rather than to any immediate reaction between the viruses themselves. Other instances, however, may be quoted in which interference seems to occur because of mutual interaction, direct or indirect, between the

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opposing viral agents. Thus, a peculiar type of resistance to superinfection has been recorded for both encephalitogenic and non-encephalitogenic strains of herpes virus in that a second intracerebral dose, following shortly after a preliminary corneal or intradermal dose, causes a mutual extinction of the effects of the two injections (7); similarly, it is said that if rabbits receive a series of intravenous injections of fixed virus after subdural infection with street virus, no rabies develops (8). Furthermore, Hoskins (9) found that intramuscular injection of a neurotropic strain of yellow fever virus, which is usually harmless for monkeys, protects these animals against simultaneous infection with a highly pathogenic viscerotropic strain of the same virus. Subsequently, Findlay and MacCallum (10) showed that the injection of a mixture of Rift Valley fever and yellow fever virus into *rhesus* monkeys served to save a majority of the animals from death by yellow fever infection; conversely, a single inoculation of mice with neurotropic yellow fever virus and pantropic Rift Valley fever virus definitely protected a few mice against the latter disease and delayed the death of others. A well marked sparing effect of the virus of lymphocytic choriomeningitis upon poliomyelitic infection in monkeys has also been described by Dalldorf and his associates (11). In all these cases interference takes place with such rapidity, and the resulting protection is limited to such brief intervals, that the failure of infection can hardly be ascribed to forces of acquired immunity, as generally denoted by this term. Moreover, in the last two instances, antagonism occurs between serologically unrelated viruses, a fact which would tend further to minimize the involvement of any specific immunological effects.

No explanation can be given, at present, for these protective phenomena. Above all, it is uncertain whether one virus acts directly upon the other so as to produce complete annihilation of both, or whether the protection is due to a restriction of viral propagation in selective cell territories which are shared by the two infectious agents. Finally it is conceivable that one virus may elaborate a soluble substance which checks the growth of the other virus. Such inhibitory substances, derived from and acting against the same virus, have already been demonstrated in tumor tissue of the Rous chicken sarcoma (12).

It will be recalled that a powerful antagonism between the murine strain of SK poliomyelitis virus and poliomyelitis monkey virus (SK and Aycock strains) was discovered previously in the course of this work (13). Further progress with this problem has recently been reported (14, 15). It is the object of this communication to present in detail the experimental basis on which these observations rest and to record the results that can be obtained in monkeys, infected with poliomyelitis virus, by administering murine virus at various stages of the disease.

EXPERIMENTAL

The experimental work is presented in three sections. The first section deals with the results obtained by the inoculation of monkeys with mixtures *in vitro* prepared of murine and poliomyelitis virus; the second with attempts to protect monkeys against poliomyelitic infection by prophylactic administration of murine virus; the third with efforts to block the course of poliomyelitis in monkeys by injecting murine virus at certain intervals following infection with monkey virus.

Results Obtained with the Intracerebral Injection of Mixtures of Murine and Poliomyelitis Virus into Rhesus Monkeys

Mixtures were prepared by combining 0.5 cc. of murine virus suspension (obtained from the brain of mice paralyzed by SK murine poliomyelitis virus) with 0.5 cc. of monkey virus suspensions (obtained from the cord of monkeys paralyzed by monkey passage poliomyelitis virus). Swiss mice, 12 to 15 gm., and *rhesus* monkeys, 1800 to 2500 gm., were used throughout this work. Immediately after their preparation these mixtures, in a volume of 1 cc., were injected intracerebrally into monkeys. Since earlier experience with tissue culture murine virus (15) had suggested that the effectiveness of interference between the opposing viruses may depend upon certain quantitative relationships, murine and monkey virus were employed in graded doses. The strains of monkey virus used in these experiments were the Aycock and the RMV virus. Tests with SK monkey poliomyelitis virus were omitted, partly because effective interference by mixing SK murine virus (mouse or culture virus) with SK monkey virus had previously been described (13, 15), and, partly because considerable difficulties were encountered in maintaining the SK strain of poliomyelitis virus at a uniformly high level of virulence in serial passages through monkeys. The specificity of the interference was determined by injecting intracerebrally into monkeys control mixtures consisting of poliomyelitis monkey virus in combination with (1) saline, (2) normal mouse brain suspensions, (3) murine virus brain suspension inactivated by heating for ½ hour at 75°C., and (4) herpetic (L.F. strain of herpes virus) mouse brain suspension. The results of these tests are brought together in Tables I and II.

It will be seen from Table I that interference between murine virus and Aycock monkey virus occurred regularly at levels of 1:10 dilution of monkey virus and 1:10 dilution of murine virus; the same dose of murine virus interfered effectively with all higher dilutions of monkey virus, except a dilution of 1:500.[1] In some monkeys a transient weakness was observed after injection with mixtures of the two viruses, particularly those containing an excess of

[1] Brain and cord of this monkey, when sacrificed at the height of paralysis, failed of transmission to monkeys with the production of paralysis, whereas transfer to mice induced paralysis. The paralysis in this monkey, therefore, may have been caused essentially by murine virus activity.

murine virus; this condition was probably caused by mouse virus activity. It will further be noted that the effectiveness of interference is gradually lost when successive dilutions of murine virus are combined with a constant dose of Aycock virus. Thus, of three monkeys receiving mixtures of Aycock virus 1:10 and murine virus 1:100 one animal developed paralysis, of three monkeys receiving mixtures of Aycock virus 1:10 and murine virus 1:1000 two animals became paralyzed, whereas neither of two monkeys injected with mixtures of

TABLE I

Interference between Murine Virus and Monkey Virus (Aycock Strain) in Mixture Experiments

Monkey virus 0.5 cc.	Murine virus 0.5 cc.	Result		
		Complete paralysis	Partial paralysis	No paralysis
1:10	1:10	0	0	1
1:100	"	0	0	1
1:500	"	1	0	0
1:1,000	"	0	0	1
1:10	1:10	0	0	2
"	1:100	1	0	2
"	1:1,000	1	1	1
"	1:10,000	2	0	0
	Controls			
1:10	Saline	1	0	0
1:50	"	2	1	0
1:10	1:10 (normal mouse brain)	1	0	0
1:100	" "	1	0	0
1:500	" "	0	1	0
1:1,000	" "	1	0	0
1:10	1:10 (heated murine virus)	1	0	0
1:10	1:10 (herpetic mouse brain)	1	0	0

Aycock virus 1:10 and murine virus 1:10,000 escaped the disease. Since all control monkeys injected with doses of Aycock virus ranging from 0.5 cc. of a 1:10 to a 1:1000 dilution succumbed to paralysis it appears that 0.5 cc. of a 1:10 dilution of murine virus was capable of protecting against at least 100 minimal paralytic doses of poliomyelitis virus. Normal mouse brain and herpetic mouse brain exercised no protective action and the interfering principle in murine virus was evidently destroyed by heating for ½ hour at 75°C.

An inspection of Table II shows that similar interference could be obtained between murine virus and RMV monkey virus, except that the lowest level of

effective interference began with a combination of RMV virus diluted 1:100 and a dilution of 1:10 murine virus; the same dose of murine virus protected effectively against all higher dilutions of RMV virus. When one estimates the degree of effectiveness of this interference and considers the fact that paralysis occurred in control animals injected with RMV virus dilutions up to 1:10,000, simple calculation shows that a dilution of murine virus of 1:10 was capable of protecting once more against at least 100 minimal paralytic doses of poliomyelitis virus.

The above findings, obtained with two different strains of monkey poliomyelitis virus, suggest that the interference between murine virus and monkey

TABLE II

Interference between Murine Virus and Monkey Virus (RMV Strain) in Mixture Experiments

Monkey virus 0.5 cc.	Murine virus 0.5 cc.	Result		
		Complete paralysis	Partial paralysis	No paralysis
1:10	1:10	1	0	0
1:100	"	0	0	1
1:200	"	0	0	1
1:500	"	0	0	1
1:1,000	"	0	0	1
1:5,000	"	0	0	1
1:10,000	"	0	0	1
	Controls			
1:200	Saline	1	0	0
1:500	"	1	0	0
1:1,000	"	0	1	0
1:5,000	"	1	0	0
1:10,000	"	1	0	0

virus proceeds on some quantitatively fixed basis. No precise formulation can be offered at this time of the actual quantitative relationships involved since virulence titrations of the two strains of poliomyelitis virus were not carried to their respective end points. However, the available data indicate that 0.5 cc. of a 1:10 dilution of murine virus will counteract at least 100 minimum paralytic doses of poliomyelitis virus, irrespective of the strain used, when such mixtures are injected intracerebrally into *rhesus* monkeys. Upon injection of apparently balanced virus mixtures not much propagation of either virus seems to occur. Thus, on one occasion in which a monkey was sacrificed on the 12th day following intracerebral injection of a non-pathogenic mixture of the two viruses, only traces of murine and of poliomyelitis virus could be recovered from brain, cord, or spleen, as determined by transfer of these tissues to mice and monkeys. Symptomless survival of monkeys following intracerebral

injection with mixtures of murine and poliomyelitis virus rarely seems to induce any permanent immunity. Thus, 4 of 5 such monkeys developed prostrating paralysis upon reinfection, 1 month later, with Aycock virus.

Results Obtained with the Administration of Murine Virus in Monkeys before Infection with Poliomyelitis Virus

At various intervals before intracerebral infection with poliomyelitis virus (RMV, Aycock) monkeys were given murine virus by the intravenous route. The murine virus was prepared by grinding three infected mouse brains in 9 cc. of tissue culture murine virus fluid, to yield a 10 per cent virus suspension. The suspension was allowed to settle for about 5 to 10 minutes and the turbid supernatant was used in amounts of 6 to 8 cc. for one intravenous dose; these injections must be given very slowly in order to avoid shock. Murine virus was given in repeated doses, varying from 3 to 5 injections, each dose being administered on successive days; the interval between the last injection of murine virus and poliomyelitic infection extended from 2 weeks to 1 day. One prophylactic series of murine virus injections, in some cases, constituted the only mode of treatment; in other cases, multiple injections of murine virus were resumed at irregular intervals following infection with poliomyelitis virus. A total of five experiments were run; in three experiments infection was produced by RMV virus[2] and in two by Aycock virus. Each experiment contained a variable number of treated monkeys and an adequate number of untreated controls, all of which were infected with the same dose of poliomyelitis virus. The results of the five experiments are listed in Table III which also gives all details concerning technical procedures.

The results obtained in the five different experiments, as given in Table III, are not strictly comparable, inasmuch as the experimental conditions varied considerably from test to test. However it appears that in the first three experiments, in which RMV virus was used, there were 3 monkeys in a treated group of 14 animals which remained entirely free from symptoms and 2 additional monkeys which developed atypical paralysis after greatly prolonged incubation periods. By contrast, all of 8 accompanying controls succumbed promptly to the disease. In the next two experiments in which Aycock virus was used a treated group of 12 monkeys included 10 animals which failed to show any paralytic symptoms whatsoever and 1 monkey which developed delayed paralysis, whereas all of 11 accompanying controls succumbed to the disease in a typical manner. In the group of treated animals which had failed to develop paralysis 6 monkeys survived sufficiently long to be reinfected, 4 weeks later, with Aycock virus. All 6 monkeys proved fully susceptible to reinfection.

In evaluating the mechanism of protection in these experiments an impres-

[2] These experiments include 14 monkeys to which reference was made in an earlier paper (16).

sion is gained that successful interference is governed not only by ensuring a proper quantitative balance between murine and poliomyelitis virus at the time of their first interaction, but also by maintenance of a continuous level of active murine virus. The existence of certain quantitative relationships between the two interfering viruses has already been demonstrated in direct mixture tests. Moreover, as shown in earlier experiments (13), murine virus

TABLE III

Interference between Murine Virus and Monkey Virus in Prophylactic Experiments

Experiment No.	No. of monkeys	Mode of murine prophylaxis		Infection with monkey virus		Result		
		Injection of murine virus	Interval between prophylaxis and infection*	Strain	Dose	Complete paralysis	Partial paralysis	No paralysis
I	2 murine	5 injections before infection	2 wks.	RMV	0.5 cc. 1:10	1 (9 days)	1 (9 days)	0
	2 "	" "	1 wk.	"	" " "	2 (9–11 ")	0	0
	2 controls	—	—	"	" " "	2 (6 ")	0	0
II	7 murine	5 injections before infection	1 wk.	RMV	0.5 cc. 1:100	3 (9–12 days)	2 (10–12 days)	2
	3 controls	—	—	"	" " "	3 (5–6 ")	0	0
III	3 murine	5 injections before infection	5 days	RMV	0.5 cc. 1:200	2 (16–19 days)	0	1
	3 controls	—	—	"	" " "	3 (7–11 ")	0	0
IV	3 murine	3 injections before infection	1 day	Aycock	0.5 cc. 1:50	0	1 (20 days)	2
	3 "	3 injections before and 4 injections after infection	1 "	"	" " "	0	0	3
	3 controls	—	—	"	" " "	2 (7–8 days)	1 (11 days)	0
V	6 murine	3 injections before and 4 injections after infection	1 day	Aycock	0.5 cc. 1:50	1 (11 days)	0	5
	8 controls	—	—	"	" " "	8 (6–12 ")	0	0
Totals	26 murine					9	4	13
	19 controls					18	1	0

* This interval denotes the time elapsed between the last injection of murine virus and infection with monkey virus.

propagates itself in the monkey only to a limited extent and is ordinarily excreted within 1 or 2 weeks. In the light of these considerations the greater success which attended the Aycock experiments as compared with the RMV experiments should cause no surprise. In the first place, the dose of Aycock virus used was not of overwhelming virulence; furthermore, some of the treated animals received additional injections of murine virus after infection with poliomyelitis virus; finally, the interval which separated the last prophy-

lactic injection of murine virus from the date of poliomyelitic infection was not over 1 day. Obviously, in all these respects, the experimental conditions were much severer in those experiments in which RMV virus was used. It remains to be seen whether equally good results can be obtained with both strains of poliomyelitis virus, provided adequate allowance is made for dosage of monkey virus, dosage of murine virus and, particularly, a more favorable spacing of intervals between injection of the two viruses.

Results Obtained by the Administration of Murine Virus to Monkeys Following Infection with Poliomyelitis Virus

The experiments described in this section were undertaken in order to determine whether the injection of murine virus in monkeys, subsequent to infection with poliomyelitis monkey passage virus, was capable of modifying or blocking the course of the disease.

Three strains of monkey virus were employed in this work, *i.e.* the SK, the Aycock, and the RMV virus. Infection with monkey virus was produced by intracerebral injection. Following infection, certain intervals were allowed to elapse before the introduction of murine virus by the intravenous route; these intervals varied from a few minutes (1st day of infection) to 96 hours (5th day of infection). The infecting doses of Aycock and RMV virus, respectively, represented at least 20 to 50 minimal paralytic doses for each strain, *i.e.* Aycock 0.5 cc. 1:10 to 1:50 dilution and RMV 0.5 cc. 1:10 to 1:200 dilution; SK monkey virus was used in amounts of 0.5 cc. 1:10 to 1:100 dilution, doses which fell within the approximate range of virulence of this particular strain. The dosage of murine virus varied widely from test to test. While no attempt was made to determine the minimum amount of murine virus which would afford protection at various intervals, some monkeys in which treatment was begun on the day of infection received one single injection only of murine virus. On the other hand, all monkeys in which treatment was delayed beyond the 1st day of infection were subjected to a series of repeated injections (5 to 9 injections). Such injections always extended over a period of several days (1 to 5 days); in some cases, two injections were given each day, in others only one dose of murine virus was administered daily. The murine virus was prepared in the same manner as previously described for the prophylactic experiments, *i.e.* a single dose consisted of three infected mouse brains, ground in 9 cc. of undiluted tissue culture virus fluid, so as to yield a final virus concentration of 1:10. Each experiment included a variable number of animals under treatment with murine virus and an adequate number of control animals. The control animals were infected with the same dose of monkey virus and, for the greater part, remained entirely free from any form of control treatment; in one experiment the controls received an equal number of intravenous injections of normal mouse brain, suspended in uninoculated tissue culture fluid instead of murine virus preparations. Three experiments were carried out with SK virus, three with RMV virus, and six with Aycock virus. The results obtained are given in Tables IV, V, and VI.

The data shown in Table IV indicate that monkeys which had received murine virus were well nigh completely protected against poliomyelitic infec-

tion induced by SK virus. This protection extended practically undiminished from the 1st day of the infection to the 96 hour interval. Thus, among a total of 23 monkeys, treated between the 1st and 5th day during the incubation period of the disease, 20 remained entirely free from paralysis, whereas of a total of 11 controls only 2 escaped paralysis. Unfortunately, the SK monkey virus in these tests failed to paralyze all of the control animals; moreover, in some of the paralyzed controls paralysis was only partial. Such marked fluctuations in the virulence of the SK monkey virus, in our experience, have

TABLE IV

Interference between Murine Virus and Monkey Virus (SK Strain) in Therapeutic Experiments

Experiment No.	No. of monkeys	Infection with monkey virus Dose	Mode of murine therapy		Result		
			Interval between infection and therapy	Injections of murine virus	Complete paralysis	Partial paralysis	No paralysi
I	1 murine	0.5 cc. 1:100	No interval	1	0	0	1
	6 "	" " "	5th day of disease	5–8	0	1	5
	4 controls	" " "	—	—	2	2	0
II	2 murine	0.5 cc. 1:100	No interval	1	0	0	2
	4 "	" " "	3rd day of disease	5–6	0	1	3
	4 "	" " "	5th " " "	5–6	0	0	4
	4 controls	" " "	—	—	1	2	1
III	3 murine	0.5 cc. 1:10	3rd day of disease	5–7	0	1	2
	3 "	" " "	5th " " "	7	0	0	3
	3 controls	" " "	—	—	0	2	1
Totals	23 murine				0	3	20
	11 controls				3	6	2

been typical of this strain of virus and render it unsuitable for critical tests. The available evidence, therefore, while suggesting that a definite therapeutic effect had been achieved, could not be regarded as entirely conclusive.

A more clear-cut picture is presented by the data given in Table V, which lists the results obtained in monkeys infected with RMV virus. It appears that of a total of 20 monkeys, treated with murine virus between the 1st and 5th day of the incubation period, 7 remained entirely free from paralysis, whereas all 9 accompanying controls succumbed to the disease. It will also be observed that 4 additional treated animals recovered with partial paralysis; on the other hand, no control animal survived the prostrating paralytic attack which is characteristic of the RMV strain. Treatment with murine virus had therefore undoubtedly afforded a considerable degree of protection against poliomyelitic infection induced by the RMV strain, particularly during the early preparalytic stage of the disease.

Essential confirmation of the results obtained with the SK and RMV strains of monkey virus can be found in the data given in Table VI which deal with the experiments in which poliomyelitic infection was produced by Aycock virus. It will be noted that of a total of 45 monkeys, treated with murine virus between the 1st and the 5th day of the disease, 24 failed to show any paralytic symptoms, whereas all 30 controls developed paralysis; among the 30 paralyzed controls paralysis was partial in only 6 animals, the remaining 24 controls succumbing to the disease with complete prostration. It should be added that among the 21 treated animals which developed paralysis (11 complete and 10 partial), there were 4 monkeys which ran an atypical course of the disease

TABLE V

Interference between Murine Virus and Monkey Virus (RMV Strain) in Therapeutic Experiments

Experiment No.	No. of monkeys	Infection with monkey virus Dose	Mode of murine therapy		Result		
			Interval between infection and therapy	Injections of murine virus	Complete paralysis	Partial paralysis	No paralysis
I	3 murine	0.5 cc. 1:10	No interval	6	0	0	3
	3 "	" " "	5th day of disease	7–8	3	0	0
	3 controls	" " "	—	—	3	0	0
II	5 murine	0.5 cc. 1:10	No interval	8–9	1	3	1
	6 "	" " "	3rd day of disease	6–9	5	1	0
	3 controls	" " "	—	—	3	0	0
III	3 murine	0.5 cc. 1:200	No interval	9	0	0	3
	3 controls	" " "	—	—	3	0	0
Totals	20 murine				9	4	7
	9 controls				9	0	0

inasmuch as paralysis occurred after greatly prolonged incubation periods (16 to 30 days). The conclusion, therefore, seems justified that the administration of murine virus in monkeys infected with the Aycock strain of poliomyelitis virus had produced distinct therapeutic effects, especially when treatment was instituted within the first 48 hours of the incubation period of the experimental disease.

When all observations relating to the treatment of experimental poliomyelitis by murine virus are considered as a whole, the combined figures of three experimental series indicate that among a total of 88 monkeys, which had received murine virus between the 1st and 5th day of the disease, 51 monkeys, or more than half (57 per cent), failed to show any paralytic symptoms, while in a group of 50 untreated controls only 2 monkeys (4 per cent) escaped the disease. By

limiting the statistical analysis to an evaluation only of the efficacy of early treatment, begun on the day of infection with poliomyelitis virus, it becomes apparent that of a total of 40 monkeys thus treated, 26 animals, or almost two-thirds (65 per cent), remained free from paralysis, as compared with one single non-paralytic survivor among 47 accompanying control animals. By contrast, when treatment was delayed until 96 hours after poliomyelitic infection, marked protection was obtained only against the weak SK strain, all treated animals infected with highly virulent RMV or Aycock virus succumbing to the disease like controls. While the above data serve as a measure of the inci-

TABLE VI

Interference between Murine Virus and Monkey Virus (Aycock Strain) in Therapeutic Experiments

Experiment No.	No. of monkeys	Infection with monkey virus Dose	Mode of murine therapy		Result		
			Interval between infection and therapy	Injections of murine virus	Complete paralysis	Partial paralysis	No paralysis
I	1 murine	0.5 cc. 1:10	No interval	1	1 (30 days)	0	0
	1 control	" " "	—	—	0	1 (9 days)	0
II	1 murine	0.5 cc. 1:20	No interval	1	1 (10 days)	0	0
	3 "	" " "	" "	6	0	0	3
	3 "	" " "	3rd day of disease	7–8	1 (7 days)	2 (10 days)	0
	3 "	" " "	5th " " "	6–7	3 (8 ")	0	0
	5 controls	" " "	—	—	4 (5–9 ")	1 (12 days)	0
III	6 murine	0.5 cc. 1:20	No interval	3–6	0	1 (7 days)	5
	3 controls	" " "	—	—	3 (7 days)	0	0
IV	4 murine	0.5 cc. 1:20	No interval	7–9	2 (9 days)	1 (10 days)	1
	4 "	" " "	3rd day of disease	7–8	0	1 (12 ")	3
	5 controls	" " "	—	—	4 (5–6 days)	1 (12 ")	0
V	5 murine	0.5 cc. 1:50	No interval	6	2 (7–8 days)	1 (16 days)	2
	5 "	" " "	3rd day of disease	6–8	0	2 (8–9 ")	3
	5 controls	" " "	—	—	3 (7–8 days)	2 (9 ")	0
VI	6 murine	0.5 cc. 1:50	No interval	9	0	1 (11 days)	5
	4 "	" " "	3rd day of disease	9	1 (20 days)	1 (20 ")	2
	11 controls	" " "	—	—	10 (6–13 ")	1 (13 ")	0
Totals	45 murine				11	10	24
	30 controls				24	6	0

dence of the disease in the treated and the control group, the effects of treatment with murine virus can also be gauged by comparing the severity of the disease in the two groups of animals. Such a comparison, whether applied to the total figures or to figures relating to early treatment, reveals that the percentage of prostrating paralysis in treated animals was always a fraction of that occurring in untreated control animals; *i.e.*, 22 per cent against 72 per cent for the total group, and 17 per cent against 82 per cent for the early treatment group. That early treatment with murine virus, when successful, virtually aborts the experimental disease, whereas delayed treatment, even though saving the animal from paralysis, is unable to prevent some form of subclinical infection, is also

strikingly demonstrated by the results of reinfection experiments. Thus, in a group of 6 surviving monkeys, in which treatment had been begun on the 1st day of infection, none escaped paralysis upon reinfection with Aycock or RMV virus; by contrast, in another group of 9 surviving monkeys, which had been treated at the 48 or 96 hour interval, 8 proved resistant and only 1 susceptible to reinfection with the same viruses.

Murine virus being capable of bringing about as powerful protective effects as would appear from these data, it may be pertinent to raise the question why it has not been possible to abort the disease in an even higher percentage of monkeys during the early stages of the incubation period. This question cannot be readily answered. As far as the fate of murine virus is concerned, it apparently persists for some time, in active form, in the central nervous system of monkeys when introduced shortly after poliomyelitic infection. For on three occasions, when monkeys injected with murine virus on the day of infection with poliomyelitis virus were sacrificed between the 1st and 7th day during the incubation period, transfers of brain and cord to mice revealed the presence of large amounts of murine virus. On the other hand, when monkeys developed poliomyelitis despite treatment with murine virus, it has usually been impossible to demonstrate any murine virus in the central nervous system of such paralyzed animals. Thus, transfers to mice of brain and cord from 4 prostrate treated monkeys, carried out at intervals of from 1 to 3 days after the last injection of murine virus, gave no evidence of the existence of any active murine virus in these tissues. All that can be said, therefore, is that the failure of interference seems to be associated with the absence of murine virus, while the data are not inconsistent with the assumption that successful interference depends upon the persistence of murine virus in active form. The lack of success in therapeutic experiments is therefore probably conditioned, partly by the existence of a proper balance between monkey and murine virus—as determined by the initial quantities of virus injected and the rate of their subsequent propagation—and partly by the maintenance of a definite threshold level of murine virus throughout the preparalytic stage of the disease. The harmonious coordination of these variables may well be materially affected by certain individual variations in the response of any given monkey to the two viruses.

DISCUSSION

The data presented in this paper show that the murine strain of SK poliomyelitis virus is capable of interfering with the development of poliomyelitic infection in *rhesus* monkeys. Murine infection in mice, on the other hand, is not significantly influenced by the administration of monkey poliomyelitis virus; nor was the growth of murine virus inhibited in tissue cultures to which monkey poliomyelitis virus had been added. This interference phenomenon, therefore, appears to be a unilateral reaction in that the stronger murine virus dominates over the weaker, simian strains. Such interference, as can be

demonstrated in monkeys, operates effectively not only against the parent SK monkey strain but also against two other highly virulent strains of monkey passage virus, *i.e.* Aycock and RMV. It is further evident that interference takes place, irrespective of whether monkey and murine virus are injected in form of *in vitro* prepared mixtures, or whether the two viruses are introduced by separate routes. When murine virus is given intravenously to monkeys before or after intracerebral infection with monkey virus, distinct prophylactic and therapeutic effects may be obtained. The limits of effective interference are set by certain critical thresholds of time and dosage which seem to govern the interaction between the two opposing viruses. Thus, the weaker culture virus makes a less effective interfering agent than highly potent mouse passage virus (15), whereas cavian passage virus, which possesses even lower virulence, has given no clear-cut evidence of therapeutically effective interference (17). It may be added that no protection occurs when intravenous injections of monkey virus are substituted for murine virus during the incubation period of the experimental disease.

The protection which is induced when murine virus interferes with the development of poliomyelitic infection in monkeys is probably not referable to any immunizing effects, humoral or cellular, of the murine strain. Thus, previous experience has demonstrated that prolonged immunization of monkeys with poliomyelitis virus, be it of simian or of murine origin, causes but rarely a state of resistance sufficiently marked to protect the immunized animal against intracerebral infection with monkey passage virus. Furthermore, protection in interference experiments is afforded in prophylactic as well as in therapeutic tests. It must also be remembered that monkeys which have survived the experience of *in vivo* interference between murine and monkey virus, as a rule, remain fully susceptible to subsequent reinfection with monkey virus. All these observations point in the direction of an immediate, though transient reaction which differs, both in its speed and lack of persistence, from classical immunological processes.

While it seems permissible to exclude immunity as being responsible for the observed protection, no explanation which pretends to have more than heuristic significance can be offered at this time for the mechanism of interference. Rivers (18) has drawn attention to a general impression, prevailing among virus workers, that unhealthy animals are either more resistant or react less severely to certain virus maladies than do perfectly healthy animals. Proceeding from this experience to a discussion of the known systems of viral interference he has suggested that normal cells might be more suitable for the multiplication of a virus than cells rendered abnormal by previous contact with another virus. This, of course, is merely a suggestion and it becomes necessary to subject the available data to a critical analysis if we expect to reach a better understanding of these obscure phenomena.

To begin with, it seems fairly obvious that the various manifestations of viral

antagonism, which, for want of a better term, have been loosely brought together under the name of "interference," make up a rather heterogeneous group in so far as their *modus operandi* is concerned. Such sparing effects, for instance, as are demonstrable between poliomyelitic and lymphocytic choriomeningitic infection, have probably no connection whatsoever with intrinsic properties of the inciting agents, since the two diseases are caused by totally unrelated viruses. The simplest explanation of the phenomenon would be to assume that poliomyelitis virus, on its way from brain to cord, is partially or completely intercepted by the barrier of an extensive inflammatory reaction which constitutes the most characteristic feature of lymphocytic choriomeningitic lesions. In other words, the failure of poliomyelitis virus to produce paralysis, in this instance, is probably due to an essentially mechanical restriction of virus to its primary site of inoculation. On the other hand, different conditions seem to obtain in those cases of viral interference in which the competing viruses represent pathogenic and non-pathogenic variants of the same strain, or are otherwise closely related. We are referring to such interference as occurs between the neurotropic and viscerotropic descendants of yellow fever virus or between the virus of yellow fever and the virus of Rift Valley fever; for even though the last two viruses and the diseases which they produce are seemingly unrelated, sufficient analogies exist to raise the question whether both viruses may not have originated from some common ancestral form (10). Obviously, the interference that is demonstrable between the pantropic murine strain of SK poliomyelitis virus and the neurotropic simian strains of poliomyelitis virus falls into the same category; and we probably do not go far astray by assuming that the basic mechanisms responsible for the several interference phenomena listed in this group are very similar. Certainly, all three types of interference just mentioned operate with singular efficiency since protection can be obtained with great regularity against multiple infecting doses of highly virulent virus. As far as information is concerned that has come to us from a study of the poliomyelitis interference system, the available data suggest that the success of interference is associated with the survival, and failure with the absence, of murine virus in the central nervous system of the monkey. It may further be taken for granted that a definite correlation exists between the potency level of murine virus, as determined by titration in mice, and its interfering ability, as tested in monkeys. Protection, therefore, seems to result from domination of one virus over the other. Precisely what the mechanism of this domination is, is impossible to say at present. It may either be mediated, in some way, by a reaction on the part of mutually susceptible cells, or else be brought about by direct virucidal interaction between the two viruses themselves. The first hypothesis seems the more plausible since both viruses, though differentially pathogenic for monkeys and mice, possess the same affinity for the anterior horn cell which constitutes the selec-

tive seat of the poliomyelitic lesion. Such a "blockade" of susceptible cells by non-paralyzing murine virus might render these cells temporarily impregnable to an attack of paralyzing monkey virus because the orderly function of certain enzyme systems, necessary for successful propagation of monkey virus, has conceivably been disturbed by previous contact with murine virus. However, it should be mentioned that positive interference has also been obtained with murine virus preparations which had been partially inactivated by exposure to ultraviolet light. Such irradiated virus, innocuous for mice by intraperitoneal injection though still mildly infectious by intracerebral test, has proven an effective interfering agent, on several occasions, in both therapeutic and mixture experiments. The question arises, therefore, whether the interfering principle in murine virus is identical with the infectious unit itself, or whether interference is brought about by a non-infectious substance, existing as an integral part of this unit or occurring separately in soluble form. It is hoped that further investigations, which are in progress, will help to throw light on this fundamental problem.

SUMMARY AND CONCLUSIONS

1. The murine strain of SK poliomyelitis virus interferes with the propagation in *rhesus* monkeys of SK, Aycock, and RMV poliomyelitis monkey virus.

2. This interference is demonstrable by intracerebral injection of mixtures of murine and monkey virus prepared *in vitro* as well as by separate injection of the two viruses by diverse routes.

3. Mixture tests carried out with graded doses of murine and monkey virus show that 0.5 cc. of a 10 per cent suspension prepared from the brains of paralyzed mice is capable of counteracting at least 100 minimal paralyzing doses of two strains of monkey virus.

4. No interference was demonstrable with suspensions of brains infected with murine virus which had been inactivated by heating for ½ hour at 75°C., or with suspensions prepared from normal mice, or with brain suspensions prepared from mice infected with herpes virus.

5. When murine virus is introduced into monkeys by the intravenous route, before or after intracerebral infection with monkey virus, distinct prophylactic or therapeutic results may be obtained.

6. Analysis of the figures shows that the success of interference depends upon (*a*) the size of the infecting dose of monkey virus, (*b*) the amount of murine virus injected, and (*c*) the choice of proper intervals between the injection of monkey and murine virus.

7. The mechanism of the interference phenomenon here described is discussed in the light of the available data.

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BIBLIOGRAPHY

1. Tyzzer, E. E., *Am. J. Path.*, 1941, **17,** 141.
2. McKinney, H. H., *J. Agric. Research,* 1929, **39,** 557.
3. McKinney, H. H., *Am. J. Bot.*, 1941, **28,** 770.
4. Kunkel, L. O., *Phytopathology,* 1934, **24,** 437. Kunkel, L. O., *Bot. Rev.*, 1935, **1,** 1. Holmes, F. O., *Phytopathology,* 1934, **24,** 845. Ainsworth, G. C., *Ann. Appl. Biol.,* 1934, **21,** 581. Caldwell, J., *Proc. Roy. Soc. Med.*, 1935, **117,** 120. Price, W. C., *Phytopathology,* 1935, **25,** 776. McWorther, F. P., *Ann. Appl. Biol.*, 1938, **25,** 254.
5. Sabin, A. B., *Brit. J. Exp. Path.*, 1934, **15,** 321.
6. Frank, S. B., *J. Inv. Dermat.*, 1938, **1,** 267.
7. Magrassi, F., *Boll. Ist. sieroterap., milan.*, 1935, **14,** 773. Magrassi, F., *Z. Hyg. u. Infectionskrankh.*, 1935–36, **117,** 501, 573. Doerr, R., and Seidenberg, S., *Z. Hyg. u. Infectionskrankh.*, 1937, **119,** 135. Doerr, R., and Kon, M., *Z. Hyg. u. Infectionskrankh.*, 1937, **119,** 679. Hallauer, C., *Z. Hyg. u. Infectionskrankh.*, 1937, **119,** 213.
8. Puntoni, V., *Ann. ig. sper.*, 1921, **31,** 27.
9. Hoskins, M., *Am. J. Trop. Med.*, 1935, **15,** 675.
10. Findlay, G. M., and MacCallum, F. O., *J. Path. and Bact.*, 1937, **44,** 405.
11. Dalldorf, G., Douglass, M., and Robinson, H. E., *J. Exp. Med.*, 1938, **67,** 333. Dalldorf, G., and Douglass, M., *Proc. Soc. Exp. Biol. and Med.*, 1938, **39,** 294.
12. Sittenfield, M. J., Johnson, B. A., and Jobling, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28,** 517. Murphy, J. B., Helmer, O. M., Claude, A., and Sturm, E., *Science,* 1931, **73,** 266.
13. Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1940, **72,** 407.
14. Jungeblut, C. W., and Sanders, M., *J. Bact.*, 1942, **43,** 85.
15. Sanders, M., and Jungeblut, C. W., *J. Exp. Med.*, 1942, **75,** 631.
16. Jungeblut, C. W., Sanders, M., and Feiner, R., *J. Exp. Med.*, 1942, **75,** 611.
17. Jungeblut, C. W., Feiner, R., and Sanders, M., *J. Exp. Med.*, 1942, **76,** 31.
18. Rivers, T. M., Lane Medical Lectures, Stanford University Publications, University Series, Medical Sciences, **4,** No. 1, Stanford University Press, 1939.